

Aphids do not attend to leaf colour as visual signal, but to the handicap of reproductive investment

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The evolution of visual warning signals is well known in animals but has received scant attention in plants. The coevolutionary hypothesis is the most influential hypothesis on warning signals in plants proposing that red and yellow leaf colours in autumn signal defensive strength to herbivores. So far, evidence in support of the hypothesis, which assumes a coevolutionary origin of autumnal leaf colours, is correlative and open to alternative explanations. We therefore tested the coevolutionary hypothesis experimentally by colouring the leaves either red or green of same-aged mountain ash (*Sorbus aucuparia*) individuals. We monitored the response of winged aphids to leaf colour using insect glue on branches with natural and artificial leaf colours in each individual. In contrast to the prediction of the coevolutionary hypothesis, aphid numbers did not differ between the individuals with artificial green or artificial red leaves. Likewise, at the within-plant level, aphids did not colonize branches with natural green leaves preferentially. However, we suggest that plants emitted warning signals because aphids colonized the hosts non-randomly. We found a strong positive correlation between aphid numbers and fruit production, suggesting an allocation trade-off between investment in plant defence and reproduction. Our study demonstrates that aphids use warning signals or cues in host selection, probably volatiles, but that they did not use leaf colour.

Keywords: herbivores; warning signal; plant–animal communication; resource allocation trade-off; autumnal leaves

1. INTRODUCTION

The evolution of visual warning signals is an extremely prolific research direction in animal communication (Ruxton *et al.* 2004), but not in the field of plant–animal interactions. Herbivory is a major selective force for plants, and defence against herbivores often entails fitness costs as indicated by allocation conflicts between defence, growth and reproduction (e.g. Koricheva 2002). Given that both herbivory and defence against attacks are costly to the plant, clear communication between plants and herbivores is important and the evolution of warning signals seems likely. Numerous

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hypotheses exist on warning signals in plants (Lev-Yadun 2006), but only a few have been successful in gaining widespread attention. A notable exception is the coevolutionary hypothesis on autumnal leaf coloration (Archetti 2000; Hamilton & Brown 2001), which stimulated a suite of theoretical and observational studies.

The coevolutionary hypothesis proposed that bright red and yellow leaves in autumn function to signal plants' defensive strength to visually oriented herbivores, particularly aphids (Archetti 2000; Hamilton & Brown 2001). The important signal is relative colour intensity, because plants compete to produce the most intense colour to avoid colonization by herbivores (Hamilton & Brown 2001). Since herbivory is most detrimental to reproductively active individuals, the related reproductive insurance hypothesis states that autumnal leaf colours are honest signals to safeguard reproduction (Sinkkonen 2006). Accordingly, only reproductively active plants use warning signals which are honest because they indicate a future drop in the levels of circulating nutrients. This process makes plants unsuitable as hosts. Field studies support the coevolutionary hypothesis by reporting a negative correlation between aphid numbers or herbivore damage and colourful leaves (Hagen *et al.* 2003; Archetti & Leather 2005; Karageorgou & Manetas 2006).

The coevolutionary hypothesis has been criticized by several articles that emphasized the many physiological functions of leaf pigments (Schaefer & Wilkinson 2004; Ougham *et al.* 2005; Schaefer & Rolshausen 2006). Leaf colour changes during autumn are a complex phenomenon with many correlated changes in leaf chemistry (Gould & Lee 2002; Ougham *et al.* 2005). Importantly, studies published in support of the coevolutionary hypothesis do not exclude alternative physiological hypotheses on autumnal leaf colours (Schaefer & Wilkinson 2004; Sinkkonen 2006) nor did they demonstrate unambiguously that aphids responded to a colour signal (Ougham *et al.* 2005). This is because field studies have been observational without controlling for correlated selection or physiological effects of leaf colours.

Here, we examine the coevolutionary hypothesis experimentally by artificially colouring the leaves either red or green in a cohort of same-aged mountain ash (*Sorbus aucuparia*) individuals and monitoring aphid colonization. The coevolutionary hypothesis predicts fewer herbivores on red-coloured individuals or branches with red leaves. We thus expected that artificially coloured red individuals bear fewer aphids than artificially coloured green individuals. Within individuals, we expected lower aphid numbers on branches with red leaves compared to those with green leaves. To exclude that applying artificial colour biases aphid host selection, we compared aphid numbers on manipulated and non-manipulated branches in green individuals. Finally, we scrutinized the core assumption of the reproductive insurance hypothesis (Sinkkonen 2006) that sucking herbivores react to reproductive investment and tested the relationship between reproduction and aphid infestation.

2. MATERIAL AND METHODS

Mountain ash sports red and yellow leaf coloration in autumn and hosts several aphid species that migrate during leaf colour changes (Blackmann & Eastop 1994). We studied a mountain ash

population of 29 individuals (less than 2.5 m high) growing on an area of 0.15 ha at 360 m a.s.l. near Freiburg (southwest Germany; 48.0°N, 7.5°E). The individuals were growing in an isolated vegetation belt, mostly in pairs with a mean distance of 4.7 m between pairs. In August 2005, we counted individual fruit set in not fully ripe fruits to exclude biases caused by fruit consumption of seed dispersers. From September onwards, two observers estimated independently the proportions of yellow, red, green and brown leaves on each individual to the nearest 5% once a week. At the beginning of October, when yellow or red leaves appeared in the population, we covered six branches (each approximately 200 cm²) of individual trees with a thin layer of Temmen insect glue (Hattersheim, Germany). Aphid infestation was calculated prior to our experiment as the number of winged aphids caught on glue-covered branches after 3 days. Individuals belonged to the species *Dysaphis sorbi* and *Rhopalosiphum insertum*.

On the same day, we coloured every second tree red and the remaining ones green so that neighbouring trees differed in colour (see figure 1 of electronic supplementary material). We used this design in order to reduce potential biases in aphid colonization caused by tree location. We used artificial instead of natural leaf colour to separate the putative leaf signal from the many concomitant and correlated changes in leaf chemistry that might influence host selection of herbivores (Ougham *et al.* 2005). We used red coloration because only red pigments are synthesized *de novo* in autumn. In each plant, we sprayed 35–60% of all leaves red or green using water-proof varnish (green, RAL 6002; red, RAL 3000; Ostendorf GmbH, Coesfeld, Germany). Using artificial instead of natural leaf colour is appropriate because it separates colour, the presumed signal, from the many correlated traits during leaf colour changes (Ougham *et al.* 2005). It thus enables us to test whether colour is a warning signal that influences host selection or whether herbivores colonize hosts in response to other signals or cues. Artificial colours resembled natural leaf coloration in mountain ash as demonstrated by reflectance spectrometry (see figure 2 of electronic supplementary material). We presume that slight differences between artificial and natural leaf colour are negligible for testing the coevolutionary hypothesis. This is because it predicts that individual colour shades are important relative to the colour of competing plants (Hamilton & Brown 2001), which, in our design, all sported either artificial red or green colours. To test for potential biases caused by artificial colour, we sprayed leaves so that in each individual three of the glue-covered branches had artificially coloured leaves and three had non-manipulated leaves. On day 3 and day 6 after colouring leaves, we removed all insects from the glue and recorded the number of winged aphids. The experiment ended at day 6 owing to leaf fall in the next 2 days.

Statistical analyses were made first on the level of individual trees. Since our primary objective was to assess the effect of experimental leaf colour on aphid numbers, we used repeated-measurement ANOVA on individual trees with total aphid number on three dates (one prior to the experiment and two afterwards) as dependent variable. We categorized fruit numbers into three categories, low, middle and high, and entered fruit categories and artificial coloration (green or red) as fixed factors into the model. Artificially coloured red and green individuals did not differ in fruit production (*t*-test: $t=1.2$, $p>0.21$). Since the proportions of artificially coloured leaves varied individually, we also investigated the relationship between overall red and yellow colours and aphid numbers with multiple regressions. To test whether aphids avoided branches with artificially coloured leaves, we compared aphid numbers on manipulated and non-manipulated branches in green individuals using paired *t*-tests. To test whether aphids actively avoided branches with red leaves within a plant, we compared aphid numbers on manipulated and non-manipulated branches (with green leaves) in red individuals using paired *t*-tests. Finally, we compared aphid infestation during the same dates in 2004 and 2005 and fruit production in both the years to assess individual consistency in both the traits.

3. RESULTS

The overall number of aphids caught on trees decreased over the 9-day time period of the experiment (figure 1). Time was the only significant within-subject factor to explain individual changes (repeated-measurement ANOVA: $F_{1,29}=64.65$, $p<0.001$). Our prediction that aphids colonize trees in response to leaf colour was not supported: neither colour nor the interactions between

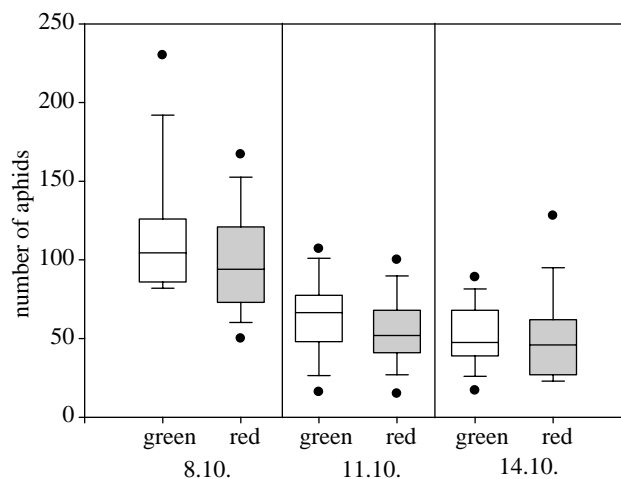


Figure 1. There was no difference in aphid numbers between individuals with artificially coloured red or green leaves prior to (8.10.) or after leaf colour manipulation (11.10. and 14.10.). Illustrated are medians, mid-quartiles and 90th and 10th percentiles and 95th and 5th percentiles as outliers.

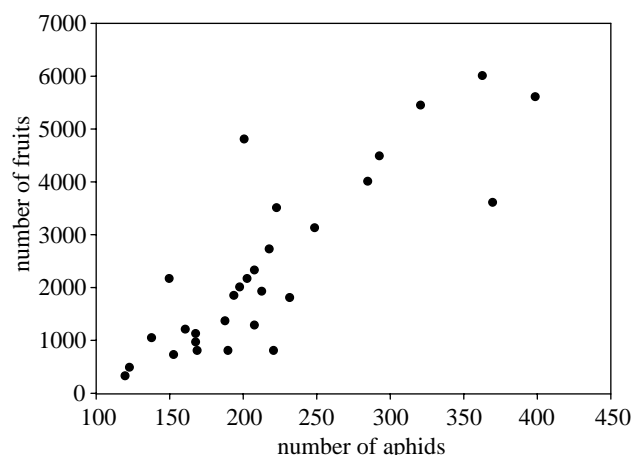


Figure 2. The number of aphids on plants correlated strongly with fruit production in mountain ash.

colour and time or number of fruits and time significantly influenced individual changes in aphid numbers during the experiment (all $F<1.26$, $p>0.1$). Likewise, leaf colour did not affect between-subject differences in aphid colonization ($F=0.2$, $p>0.5$) and there was no relationship between the proportions of red or yellow leaves and the aphid numbers (multiple regressions for both dates: $F<0.5$, $p>0.5$). Instead, differences in the number of aphids caught on individual trees were explained by the differences in the fruit numbers ($F_{1,29}=16.25$, $p<0.01$). Fruit production and total aphid infestation were strongly correlated ($r=0.83$, $p<0.001$; figure 2), apparently indicating an allocation conflict between the reproductive investment and the level of plant defence. Comparing fruit production and aphid infestation rates in 2004 and 2005 showed no difference between years ($t=0.5$ and 0.6 , both $p>0.62$) and a high degree of individual consistency in both traits ($r=0.42$, $p<0.05$ and $r=0.57$, $p<0.01$, respectively). Fruit numbers and leaf coloration were not correlated ($r=0.26$, $p>0.19$).

During both time-intervals of the experiment, aphid numbers did not differ between branches with non-manipulated and manipulated leaves in trees

with green artificial colour (*t*-test: $t = -0.45$ and 1.12 , both $p > 0.27$). Likewise, there was no difference in aphid numbers between branches with manipulated and non-manipulated leaves in red coloured individuals (*t*-test: $t = -1.64$ and -0.39 , both $p > 0.15$).

4. DISCUSSION

Artificial red and green leaf colours did not influence aphid colonization rates in mountain ash. Thus, our results do not support the core assumption—that leaf colour functions as a signal to aphids—of both the coevolutionary hypothesis and the reproductive insurance hypothesis. The strong positive correlation between fruit production and aphid load documents instead that individuals with higher reproductive investment are preferentially attacked. This result corresponds to the scenario that the reproductive insurance hypothesis predicts in the *absence* of a warning signal that informs herbivores about the future unsuitability of the host. We suggest that host selection by aphids is mediated by a trade-off between reproduction and investment in plant defences in mountain ash.

The principle of allocating limited resources to growth, reproduction and defence presumably shapes the evolution of plant defences (reviewed in Strauss *et al.* 2002). This paradigm of resource allocation conflicts predicts that investment in reproduction reduces plant defences; a scenario that is consistent with the positive correlation between fruit numbers and aphid infestation in our study. Alternatively, sucking insects might congregate on reproductively committed trees because they transport high nutrient levels to developing seeds (Sinkkonen 2006). If winged aphids select hosts according to ongoing reproduction, they might optimize performance of sexuales, which benefit from elevated nutrient levels.

High consistency in the relationship between individual plant quality and aphid infestation in two years suggests that host selection is mediated by warning signals or inadvertently transmitted cues. However, in contrast to the assumption of the coevolutionary hypothesis and the reproduction insurance hypothesis, the warning signal is not a visual one. Artificial colours are commonly used to assess site selection by winged aphids (Döring *et al.* 2004). Here, we show that aphids did not colonize plants according to (artificial) leaf colour. We consider it unlikely that our result is caused by biases owing to artificial colour, because aphids did not distinguish between leaves on manipulated and non-manipulated branches. In addition, individual aphid infestation during manipulation did not differ from that of the previous year during the same period. Another potential limitation of our experiment is the relative short duration, but the time span covered the peak of aphid migration. In 2004, 45% of aphids that migrated during leaf fall were caught during these days (Rolshausen & Schaefer *in press*). The situation was probably similar in 2005 because we started the experiment when leaves began to change colour and it ended when non-manipulated and manipulated leaves were shed.

In sum, we propose that aphids did not use visual warning signals in host selection, which is consistent

with the environmental control of leaf coloration in mountain ash (Rolshausen & Schaefer *in press*). Aphids might have used an olfactory warning signal of those individuals that bear few fruits, but have high defences or simply attended to the visual cue of brightly coloured fruits. To understand whether coevolution explains plant–herbivore communication, it is thus pivotal to differentiate whether signals or cues govern host selection in aphids. If plants use warning signals, we emphasize that resource allocation conflicts provide the theoretical framework to interpret their evolution and suggest that future research should focus on olfactory warning signals which might explain negative correlations between leaf coloration and aphid numbers in previous studies (Rolshausen & Schaefer *in press* and others studies). Careful experiments are needed to separate signals from the many correlated physiological changes during leaf senescence. Leaf colour changes are apparently one of these correlated changes that, although linked to plant defence (Schaefer & Rolshausen 2006), did not result in differential host selection in mountain ash.

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